

Listing of the Claims

1. (Previously presented) A method for producing a protein of interest comprising:
 - a) providing:
 - i) at least one host cell comprising a genome, wherein said host cell has an immortal phenotype, and
 - ii) a plurality of retroviral vectors comprising 5' and 3' long terminal repeats, wherein said retroviral vectors encode a gene of interest operably linked to an exogenous promoter internal to said 5' and 3' long terminal repeats; and
 - b) contacting said at least one host cell with said plurality of retroviral vectors under conditions such that said host cells are transduced to produce transduced host cells, wherein said conditions comprise contacting said host cell at a multiplicity of infection of from about 10 to 1000;
 - c) repeating steps a) and b) a plurality of times
 - d) clonally selecting a host cell expressing said gene of interest, wherein the genome of said host cell comprises from 20 to 100 integrated retroviral vectors; and
 - e) purifying a protein of interest encoded by said gene of interest.
2. (Original) The method of Claim 1, wherein steps a and b are repeated at least 3 times.
3. (Original) The method of Claim 1, wherein steps a and b are repeated at least 4 times.
4. (Original) The method of Claim 1, wherein steps a and b are repeated at least 5 times.
5. (Original) The method of Claim 1, wherein steps a and b are repeated at least 6 times.
6. (Original) The method of Claim 1, wherein steps a and b are repeated at least 7 times.
7. (Original) The method of Claim 1, wherein steps a and b are repeated at least

8 times.

8. (Original) The method of Claim 1, wherein steps a and b are repeated at least 10 times.

9. (Original) The method of Claim 1, wherein steps a and b are repeated at least 20 times.

10. (Original) The method of Claim 1, wherein steps a and b are repeated between about 3 and 20 times.

11. (Cancelled).

12. (Previously presented) The method of Claim 1, wherein said retroviral vectors utilized in steps a and b are produced from packaging cells transfected with an envelope plasmid and a vector plasmid.

13. (Cancelled).

14. (Original) The method of Claim 12, wherein said packaging cells express retroviral gag and pol proteins.

15. (Original) The method of Claim 14, wherein said packaging cells are 293-GP cells.

16. (Original) The method of Claim 12, wherein said envelope plasmid encodes a G protein.

17. (Original) The method of Claim 16, wherein said G protein is VSV-G protein.

18. (Original) The method of Claim 1, wherein said retroviral vector comprises MoMLV elements.

19. (Cancelled).

20. (Original) The method of Claim 1, wherein said gene of interest is operably linked to an exogenous promoter.
21. (Previously presented) The method of Claim 1, wherein gene of interest is operably linked to a secretion signal sequence.
22. (Original) The method of Claim 1, wherein said retroviral vector encodes at least two genes of interest.
23. (Original) The method of Claim 22, wherein said at least two genes of interest are arranged in a polycistronic sequence.
24. (Original) The method of Claim 23, wherein said at least two genes of interest comprise immunoglobulin heavy and light chains.
25. (Original) The method of Claim 1, wherein said retroviral vector is a lentiviral vector.
26. (Previously presented) The method of Claim 1, wherein said host cell is selected from Chinese hamster ovary cells and human 293 cells.
27. (Cancelled).
28. (Previously presented) The method of Claim 1, further comprising culturing said clonally selected host cells under conditions such that a protein of interest encoded by said gene of interest is produced.
29. (Cancelled).
30. (Original) The method of Claim 28, further comprising isolating said protein of interest.
31. (Original) The method of Claim 28, wherein said culture conditions are selected from the group consisting of roller bottle cultures, perfusion cultures, batch fed cultures, and petri dish cultures.

32. (Previously presented) The method of Claim 28, wherein said clonally selected host cells synthesize greater than about 1 picograms per cell per day of said protein of interest.

33. (Previously presented) The method of Claim 28, wherein said clonally selected host cells synthesize greater than about 10 picograms per cell per day of said protein of interest.

34. (Previously presented) The method of Claim 28, wherein said clonally selected host cells synthesize greater than about 50 picograms per cell per day of said protein of interest.

35. (Original) The method of Claim 1, wherein said retroviral vector further encodes an amplifiable marker.

36. (Original) The method of Claim 35, wherein said amplifiable marker is selected from the group consisting of DHFR and glutamine synthetase.

37. (Original) The method of Claim 35, further comprising the step of culturing said transduced host cells under conditions that allow for amplification of the integrated retroviral vectors.

38. (Original) The method of Claim 37, wherein said conditions comprise culturing said transduced host cells in the presence of a selection agent selected from the group consisting of methotrexate, phosphinothricin and methionine sulfoxime.

39. (Previously presented) The method of Claim 24, wherein said immunoglobulins are selected from the group consisting of IgG, IgA, IgM, IgD, IgE and sIg.

40. (Original) The method of Claim 1, wherein said host cell is transduced with at least two different vectors encoding different genes of interest.

41. (Previously presented) A host cell produced by the method of Claim 1,

wherein said host cell comprises from 20 to about 100 integrated retroviral vectors.

42. (Cancelled).